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Published in:
Plant Physiology

DOI:
10.1104/pp.16.01804

Publication date:
2017

Document Version
Peer reviewed version

Plant Physiology Preview. Published on March 7, 2017, as DOI:10.1104/pp.16.01804

RXLR effector AVR2 up-regulates a brassinosteroid responsive bHLH transcription factor to suppress immunity

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Author Contributions

D.T., S.N., P.E.H., J.Z., P.R.J.B and E.M.G conceived of and designed the experiments. D.T., L.Y., S.N., S.B., L.W., J.S., J.M., P.C.B., E.M.G performed the experiments. D.T., L.Y and S.N performed most of the experiments. L.W provided technical assistance to E.M.G. J.M provided technical assistance to P.E.H. D.T., L.Y., S.N., P.E.H., P.R.J.B and E.M.G analysed and interpreted the results. D.T., S.N., P.R.J.B and E.M.G wrote the paper with contributions from other authors. P.R.J.B and E.M.G directed the project.

Funding

This work was funded by the Scottish Government Rural and Environmental Science and Analytical Services Division (RESAS), the Genomia Fund, part of the European Regional Development Fund with financial support of KWS SAAT SE and the Biotechnology and Biological Research Sciences Council (BBSRC: projects BB/G015244/1, BB/K018183/1, BB/L026880/1). PhD student D.T was funded on BBSRC CASE Award Agreement 3379_CRT, PhD student L.Y. was supported by funding from the China Scholarship Council (CSC).

Acknowledgements

This work was funded by the by the Scottish Government Rural & Environment Science & Analytical Services Division (RESAS) and the Biotechnology and Biological Sciences Research Council (BBSRC) through the projects BB/G015244/1, BB/K018183/1, BB/L026880/1. PhD student D.T. was the recipient of a BBSRC CASE studentship and L.Y. is supported by funding from the China Scholarship Council (CSC). Additional funding support was obtained from the James Hutton Institute SEEDCORN initiative. We also thank members of the Dundee Effector Consortium and our close collaborator Dr. Frédéric Brunner for helpful discussions during this work**.** We thank all members of the FUNGEN team at the James Hutton Institute for generating the 35S:Avr2 potato cv. Desiree transgenics.Thank you to Hazel McLellan for proof reading of the manuscript.

ABSTRACT

An emerging area in plant research focuses on antagonism between regulatory systems governing growth and immunity. Such crosstalk represents a point of

vulnerability for pathogens to exploit. AVR2, an RXLR effector secreted by the potato blight pathogen *Phytophthora infestans,* interacts with potato BSL1, a putative phosphatase implicated in growth-promoting brassinosteroid (BR) hormone signalling. Transgenic potato (*Solanum tuberosum)* plants expressing the effector exhibit transcriptional and phenotypic hallmarks of over-active BR signalling, and show enhanced susceptibility to *P. infestans*. Microarray analysis was used to identify a set of BR-responsive marker genes in potato, all of which are constitutively expressed to BR-induced levels in AVR2 transgenic lines. One of these genes was a bHLH transcription factor, designated StCHL1, homologous to AtCIB1 and AtHBI1 which are known to facilitate antagonism between BR and immune responses. Transient expression of either AVR2 or CHL1 enhanced leaf colonisation by *P. infestans* and compromised immune cell death activated by perception of the elicitin INF1. Knockdown of *CHL1* transcript using Virus-Induced Gene Silencing (VIGS) reduced colonisation of *P. infestans* on *Nicotiana benthamiana*. Moreover, the ability of AVR2 to suppress INF1-triggered cell death was attenuated in *NbCHL1* silenced plants, indicating that NbCHL1 was important for this effector activity. Thus AVR2 exploits crosstalk between BR signalling and innate immunity in *Solanum* species, representing a novel, indirect mode of innate immune suppression by a filamentous pathogen effector.

Introduction

Plants are sedentary and cannot escape the challenges they sense in their environment. In order to best utilise the available resources, plants are equipped with a large number of receptor-like proteins linked to complex networks of interacting signal transduction pathways that allow them to respond appropriately and rapidly to environmental conditions. Plants can detect a multitude of potential invaders, including bacteria, fungi and oomycetes and have evolved two key inducible mechanisms by which they can defend themselves. In the first

instance, Microbe- or Pathogen-Associated Molecular Patterns (MAMPs/PAMPs) can be detected by Pattern Recognition Receptors (PRRs). PRRs initiate Pattern-Triggered Immunity (PTI) and provide broad spectrum disease resistance, often to whole classes of micro-organisms (Jones and Dangl, 2006). Many pathogens deliver 'effectors' – specialised proteins which act outside or within plant cells to suppress immunity, or modify other host processes to increase disease potential. Effectors in turn may be recognised by corresponding resistance (R) proteins, activating a rapid immune response known as effector triggered immunity (ETI), which frequently results in a localised cell death known as the hypersensitive response (HR) (Jones and Dangl, 2006; Feechan et al, 2015).

Although there is some evidence in the literature indicating that growth and defence can be regulated simultaneously (Francisco et al., 2016; Campos et al., 2016; de Wit et al., 2013), much of the research done in the area of growth and immunity has shown that activation of plant defence responses requires a major re-allocation of resources away from growth to immunity (Huot et al., 2014). Consequently, plants must tightly regulate and fine-tune the signals that control this trade-off. This compromise between growth and defence is controlled at multiple levels, and shown to depend on the action of several plant hormones, including jasmonates (JA), gibberellins (GA), brassinosteroids (BR), and salicylic acid (SA) (Albrecht et al., 2012; Belkhadir et al., 2012; Yang et al., 2013; Lozano-Durán et al., 2013; Chandran et al., 2014; Wang and Wang, 2014; Fan et al., 2014; Malinovsky et al., 2014). Recent work has particularly focused on antagonistic crosstalk between the BR signalling pathway, involved in growth and development, and aspects of plant immunity (Jiménez-Góngora et al., 2015; Albrecht et al., 2011, Belkhadir et al., 2011). The BR pathway is well characterised in Arabidopsis and is essential in growth and development by regulating cell expansion, vascular differentiation, etiolation and reproductive development. Consequently, plants insensitive to BR or unable to generate BRs are acutely dwarfed, exhibiting small, dark green leaves with severe defects in cell division and elongation (Zhiponova et al., 2013).

The current understanding of the Arabidopsis BR pathway is potentially over-simplified, as every regulatory step may involve a number of closely related but less well characterised family members, paralogues and splice variants (Mora-Garcia et al., 2004; Maselli et al., 2014; Wang and Mao, 2013; Zhang et al., 2014). In the current model, BRs bind directly the LRR-receptor like kinase (RLK) BR Insensitive 1 (BRI1) (Li and Chory, 1997). This induces BRI1 dimerization, hetero-oligomerisation with BRI1-ASSOCIATED KINASE 1 (BAK1) and release of the negative regulators BRI1 Kinase Inhibitor 1 (BKI1) and Botrytis-Induced Kinase1 (BIK1) (Lozano-Durán and Zipfel, (2015); Heese et al., (2007); Nam & Li (2002). BRI1 activity causes successive phosphorylation and activation of the receptor-like cytoplasmic kinases (RLCKs) BR Signalling Kinases (BSKs) and Constitutive Differential Growth1 (CDG1) (Kim et al., 2009; Kim et al., 2011, Tang et al., 2008; Sreeramulu et al., 2013) followed by interaction with BRI1 Suppressor 1 (BSU1). BIN2 inactivation allows the accumulation of the transcription factors Brassinazole-Resistant 1 (BZR1) and BRI1-Ems-Suppressor 1 (BES1). Upon accumulation, the transcription factors, BZR1 and BES1, undergo dephosphorylation by the protein phosphatase PP2A, which allows them to be relocated to the nucleus where they orchestrate the expression of BR-responsive genes (Kim and Wang, 2010). Previously reported transcriptional changes include the up-regulation of expansins and cell-wall modifying genes, regulation of other plant hormone pathways and light signalling (Mussig et al., 2002; Goda et al., 2002).

Perception of brassinosteroid has been shown to suppress PTI; initially hypothesised to be the result of competition for BAK1 between competing RLKs, the flagellin recognising receptor, FLS2 and BR receptor, BRI1 (Belkhadir et al., 2012). However, Albrecht et al., (2011) showed that BR perception had no effect on the amount of BAK1 available for interaction with FLS2. Furthermore, while BAK1 has been shown to play no role in chitin perception (Schwessinger et al., 2011), treatment with exogenous BR inhibited the ROS burst associated with chitin perception by Chitin Elicitor Receptor Kinase 1 (CERK1),

indicating that the link between BR perception and immune signalling is not solely due to this shared co-receptor (Albrecht et al., 2011). More evidence points towards conflict between transcriptional regulators of both pathways. Recently the interaction and phosphorylation of BES1 by the PTI activated Mitogen-Activated Protein Kinase (MAPK), MPK6, has been identified as a possible mechanism of PTI induced inhibition of BR signalling (Kang et al., 2015). Furthermore, BZR1 has been demonstrated to be a central regulatory component in the cross-talk between growth and development (Lozano-Durán *et al*., 2013). BZR1 is proposed to be linked to the BR-dependent induction of expression of the bHLH (basic helix-loop-helix) transcription factors, Cryptochrome-Interacting Basic-Helix-Loop-Helix 1 (CIB1), BR Enhanced Expression 2 (BEE2) and Homolog of BEE2 Interacting with IBH 1 (HBI1), and that act partially as negative regulators of PTI in Arabidopsis (Lozano-Durán *et al*., 2013; Malinovsky et al., 2014). AtHBI1 has been best characterised, acting as both a positive regulator of BR-responses (Bai et al., 2012) and a negative regulator of immunity (Fan et al., 2014).

A broad range of host targets and activities have been elucidated for pathogen effectors secreted into host plant cells. Many effectors act on positive regulators of immunity in order to inhibit their activity (Whisson et al 2016, Deslandes and Rivas, 2012; Dou and Zhou, 2012; Feng et al., 2012; Block and Alfano, 2011;). In contrast, a number of pathogen effectors have been found to target host proteins that negatively regulate immunity (Yang et al., 2016; Boevink et al., 2016a; Wang et al., 2015; Cui et al., 2010; Chen et al., 2010). Negative regulators in the host that are required by pathogens to aid disease progression, and are thus manipulated by effectors to promote or use their activity, have been designated as susceptibility (S) factors (van Schie and Takken, 2014; Boevink et al., 2016b).

The cytoplasmic RXLR-EER effector from *Phytophthora infestans*, AVR2, accumulates in the pathogen at the site of haustorium formation, is upregulated during the biotrophic phase of

infection on potato (*Solanum tuberosum*) and is recognised inside plant cells by the host resistance protein R2 (Gilroy et al., 2011a). A kelch-repeat containing phosphatase; StBSL1 (BRI1 Suppressor 1 (BSU1)-Like 1), was identified as an interactor of AVR2 in potato. Although silencing of *BSL1* in *Nicotiana benthamiana* perturbed recognition of AVR2 by R2 family members, there was no apparent developmental phenotype or impact on susceptibility to *P. infestans* (Saunders et al., 2012). StBSL1 is homologous to one of the four members of the BSU1 family known in Arabidopsis. Interestingly, the knockout mutants of *BSU1* or *BSL1* in Arabidopsis are also phenotype-neutral (Mora-Garcia et al., 2004). Most evidence of the role of this phosphatase family in the BR pathway has been generated by studying BSU1, which is weakly expressed in mature leaves and has recently been shown to be a *Brassicaceae*-specific family member (Mora-Garcia et al., 2004; Maselli et al., 2014).

To investigate the role of pathogen effector AVR2 in late blight development, we generated transgenic potato plants that stably express this effector. We observed that AVR2 transgenic lines exhibited developmental and transcriptional changes that are hallmarks of BR pathway activation, and showed enhanced susceptibility to *P. infestans*. One transcript (bHLH7) up-regulated by BR treatment, and constitutively expressed in AVR2 lines, was of particular interest as it shares homology with transcription factors AtCIB1 and AtHBI1, shown to regulate crosstalk between PTI and BR signalling (Fan et al., 2014; Malinovsky et al., 2014; Bai et al., 2012). We utilised Agrobacterium–mediated transient expression of AVR2 and StCIB1/HBI1-like1 (StCHL1) to assess their impact on susceptibility of *N. benthamiana* to *P. infestans* and the BAK1-dependent responses to the oomycete PAMP elicitin INF1 (Du et al., 2015; Derevnina et al., 2016). We further evaluated the role of *NbCHL1* by VIGS in *N. benthamiana* and demonstrated that the ability of AVR2 to suppress INF1-triggered cell death is at least partially dependent on CHL1.

Results and Discussion

Transgenic potato lines expressing AVR2 display BR-associated developmental phenotypes

Transgenic *S. tuberosum* cv. Desiree lines were generated with constitutive, 35S-promoter-driven AVR2 expression. Two lines were taken forward for further analysis (#29, #39) to investigate the impact of AVR2 on BR signalling. Critically, in contrast to untransformed cv. Desiree, when the resistance gene *R2* was transiently expressed in leaves both AVR2 transgenic lines responded with a clear HR confirming the presence of a recognised AVR2 protein (Supplementary Figure S1). 35S:AVR2 plants displayed a variety of developmental phenotypes including twisted stems, with curled leaflets (Figure 1a, b). Compound leaf formation was also affected, with reduced numbers of leaflets, and organ fusion where leaflets failed to separate was evident. Petioles and leaves also exhibited loss of symmetry, and tended to extend from the main stem in a curved manner (Figure 1a, b). These phenotypes are reminiscent of BR-overactive phenotypes in Arabidopsis generated by *BRI1* and *DWF4* overexpression (Wang et al., 2001), *bik1* mutation (Lin et al., 2013) and *BZR1* overexpression (Gendron et al., 2012). Visualising 35S:AVR2 potato leaves under the microscope revealed increased epidermal cell size (Supplementary Figure S2). Additionally, stomatal frequency was found to be significantly decreased (Figure 1c, d), consistent with the decreased stomatal frequency observed when BSL family members are over-expressed in Arabidopsis (Kim et al., 2012). This evidence suggests that AVR2 may activate BR responses in potato. BR-responsive gene expression markers were next sought to confirm this.

BR-responsive genes are upregulated in AVR2 expressing potatoes

To understand the impact of brassinosteroid signalling on the potato transcriptome, and to identify a set of BR marker genes relevant to this crop species, microarray analysis of BR-

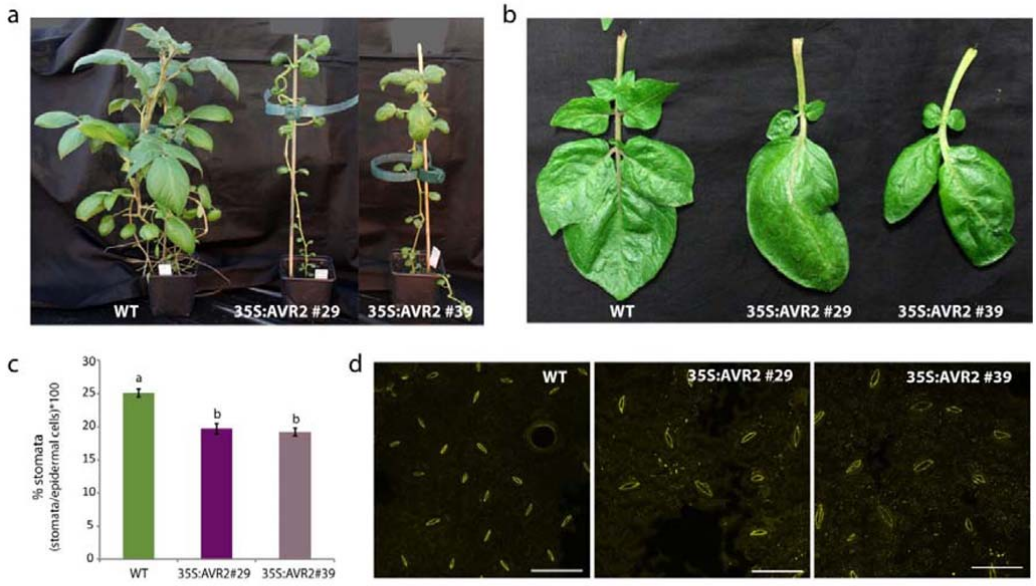
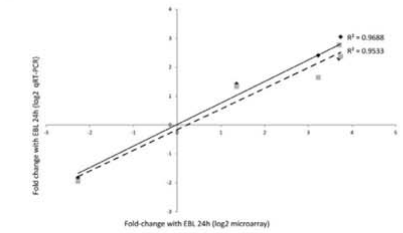


Figure 1 Transgenic potato cv. Desiree lines expressing 35S:Avr2 show morphological hallmarks of an overactive BR pathway. (a) Growth morphology of 35S:AVR2 plants (#29 and #39) showing twisted stems and curled leaves, compared to untransformed potato cv. Desiree (WT). (b) Leaf phenotype of 35S:AVR2 plants showing reduced compound formation and loss of symmetry. (c) Reduced percentage of stomata in 35S:AVR2 potato plants. Stomata count was expressed as % of total epidermal cells counted per 500 µm. Results combine three biological replicates, each consisting of epidermal leaf prints from three or more plants. Error bars indicate SEM; letters denote significant difference ($p<0.001$ in one-way ANOVA, Holm-Sidak). (d) Confocal microscopy showing reduced stomatal frequency in 35S:AVR2 potato, and enlargement of stomata relative to WT plants. Images are of representative leaves stained with calcofluor white. Scale bar = 100um

1

treated *S. tuberosum* cv. Desiree was performed. The top fifty differentially expressed genes at 3 and 24 hrs after foliar spray with the brassinosteroid epibrassinolide (EBL) compared to water sprayed controls are shown in Supplementary Tables S1-S4. Five marker genes were

a



b

				expression fold-change with EBL 24h		
Microarray ID	Gene number	Gene name	UniRef based functional annotation	Micro-array	qRT-PCR	qRT-PCR (2)
CUST_44514_PI426222305	PGSC0003DMG400016695	StCAB50	Chlorophyll a-b binding protein 50	13.368	8.301	5.193
CUST_46827_PI426222305	PGSC0003DMG400000711	StCHL1	BHLH7	13.035	4.936	6.845
CUST_44325_PI426222305	PGSC0003DMG400003912	StP69F	P69F protein	9.366	5.321	3.131
CUST_43185_PI426222305	PGSC0003DMG400016561	StSAUR67	Auxin-induced SAUR	2.561	2.682	2.320
CUST_24559_PI426222305	PGSC0003DMG400021095	StGA2ox1	Gibberellin 2-oxidase 1	0.208	0.281	0.258

c

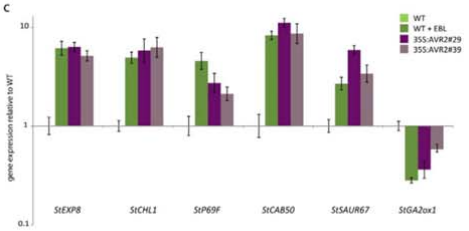


Figure 2 Microarray analysis of brassinosteroid (EBL treatment) response in potato (*Solanum tuberosum*) cv. Desiree. (a) Microarray validation by qRT-PCR of two independent biological replicates plotted in one graph. Fold-change from microarray data plotted against fold-change from qRT-PCR for 5 selected BR marker genes examined at 24 hpt. Fold-change log2 transformed to allow symmetry of up and down-regulation. Linear regression was used to determine a co-efficient of determination (R^2). (b) Table of selected marker genes showing significant differential expression with BR treatment. Fold-change values are shown from the microarray data, qRT-PCR validation, and an independent biological replicate (c) Relative expression of brassinosteroid-regulated genes in untreated potato cv. Desiree (WT; given a value of 1), WT at 24 h after treatment with EBL; and constitutive levels of expression in 35S:AVR2 potato plants, assessed by qRT-PCR. Expression was normalised to *StUbi* and shown relative to WT untreated plants. Graph shows the average of three technical replicates +/- standard deviation, with similar trend observed in two independent biological replicates.

selected from the microarray dataset (four up-regulated and one down-regulated), validated by qRT-PCR, and observed to be similarly differentially expressed following EBL treatment in independent biological replicates (Figure 2a). Amongst these were three of the fifty most

highly induced genes following EBL treatment (Supplementary Table S1): *CAB50*, encoding a chlorophyll a-b binding protein associated with light harvesting; P69F, encoding a subtilisin-like proteinase; and a basic helix-loop-helix transcription factor (bHLH7) with significant blast hit matches to two closely related bHLH transcription factors (TFs), CIB1 and HBI1 from Arabidopsis (Figure 2b; Supplementary Figures S3 and S4). The potato bHLH7 sequence was thus renamed StCIB1/HBI1-like 1 (StCHL1). AtCIB1 and AtHBI1 are known to play a role in cell elongation and responses to BR (Bai et al., 2012). Another sequence upregulated by 2 to 7-fold by BR treatment had high sequence homology to a BR inducible member (SAUR67) of the SAUR (SMALL AUXIN UPREGULATED RNA) family (Spartz et al., 2012). GIBERELLIN-2-OXIDASE1 (StGA2ox1), an enzyme involved in catalysis, or breakdown of endogenous gibberellic acid (GA) in plants (Lo et al., 2008; Bai et al. 2012) was down-regulated around 5-fold by BR in this microarray. We also designed qRT-PCR primers to a candidate potato orthologue (reciprocal best BLAST hit) of a known BR-upregulated marker gene *EXP8* in Arabidopsis (Malinovsky et al., 2014; Bai et al., 2012), *StEXP8,* which was not detected as significantly upregulated in our microarrays, was confirmed to be upregulated using qRT-PCR. These six markers were used to assess BR pathway activity in 35S:AVR2 potato lines #29 and #39. Increased transcript accumulation of the marker genes *CAB50, P69F, StCHL1, StSAUR67, and StExp8* following EBL treatment in untransformed potato reached levels similar to their constitutive expression in 35S:AVR2 potato lines, whereas EBL treatment down-regulated *StGA0x1* to levels similar to those in untreated 35S:AVR2 potato lines (Figure 2c). This supports the phenotypic observations that these AVR2 lines display constitutive BR pathway over-activity. Future microarray analyses will reveal the global transcriptional changes resulting from AVR2 expression in these transgenic lines.

PTI down-regulates BR-responsive genes in Potato

248 The crosstalk between PTI and BR identified in Arabidopsis would predict that our BR
249 response marker genes in potato should also be down-regulated by PAMP treatments
250 (Jiménez-Gongora et al., 2015). Consequently, the expression of BR responsive genes was
251 examined by qRT-PCR following treatment of WT potato cv. Desiree with the bacterial
252 flagellin-derived PAMP flg22, and with *P. infestans* culture filtrate (CF), which can be
253 regarded as a cocktail of *P. infestans* PAMPs (McLellan et al., 2013). As anticipated, in
254 contrast to the PTI marker genes *StWRKY7* and *StACRE31* (McLellan et al., 2013; Nguyen
255 et al., 2010), the BR marker genes were actively down-regulated following flg22 and CF
256 treatments, as were additional genes, *StDWF5* and *StSTDH,* associated with BR
257 biosynthesis (Figure 3a, b). Recently, AtHBI1 has been implicated as a crosstalk regulator
258 between PTI and BR-associated growth and may act redundantly with CIB1 and BEE2, all of
259 which are down-regulated by PAMPs in Arabidopsis (Malinovsky et al., 2014; Fan et al.,
260 2014, Bai et al., 2012). Our finding supports the existing observation that BR-responsive
261 genes are down-regulated during PTI in Arabidopsis (Jiménez-Gongora et al., 2015); by
262 demonstrating that this antagonism also occurs in the *Solanaceae*.
263
264 **Avr2 transgenic plants are more susceptible to *P. infestans***

265 To further investigate the impact of AVR2 expression in potato, transgenic 35S:Avr2 lines
266 and wild type (WT) Desiree controls, were inoculated with a concentrated suspension of *P.*
267 *infestans* sporangia. At 7 days after inoculation, a significant increase was observed in *P.*
268 *infestans* lesion size on leaves of the AVR2 transgenic lines, compared to the WT potato
269 (Figure 4a, b), indicating that these plants, in addition to exhibiting phenotypes of
270 brassinosteroid over-activity and constitutive expression of BR-responsive marker genes,
271 are also more susceptible to late blight.
272

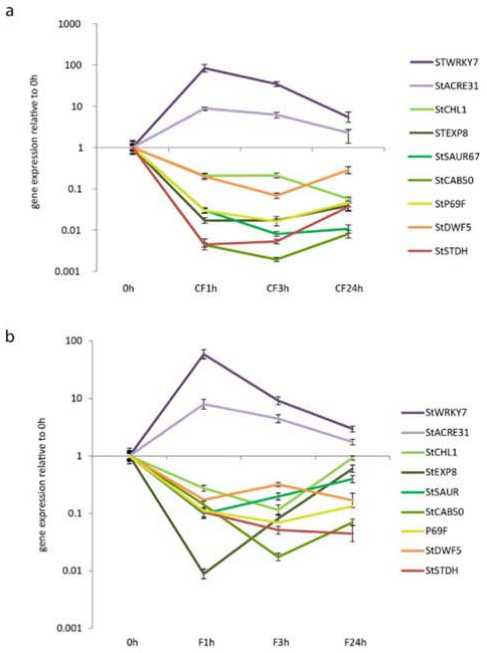


Figure 3 Brassinosteroid-responsive genes are suppressed by PTI. (a) Treatment of potato cv. Desiree with *P. infestans* culture filtrate (CF) results in transcript accumulation of PTI marker genes *StWRKY7* and *StACRE31* by 1 hour after treatment (CF1h), but reduced transcript abundance of BR (EBL)-induced genes *StCHL1*, *StEXP8*, *StSAUR67*, *StCAB50* and *StP69F*, and of brassinosteroid biosynthesis-associated genes *StDWF4* and *StSTDH*. (b) Treatment of potato cv. Desiree with the bacterial PAMP flg22 results in the same opposing patterns of transcript abundance by 1 hour after treatment (F1h) for PTI, BR and brassinosteroid biosynthesis markers as observed in (a). Error bars represent SD across 3 technical replicates, with similar trend observed in two independent biological replicates.

Expression of AVR2 suppresses INF1-triggered cell death and enhances *P. infestans* colonisation in *N. benthamiana*

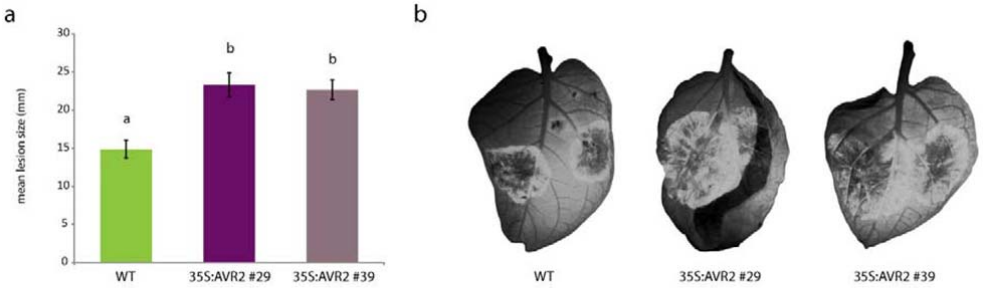


Figure 4 AVR2 expression in potato results in increased susceptibility to *P. infestans*. (a) Lesion size of transgenic *P. infestans* isolate 88069 expressing tdTomato (McLellan *et al*. 2013) (diameter in mm) on 35S:AVR2 potato at 7 days post inoculation of sporangia suspension. Data shown combines 2 independent replicates, each comprising 10 or more leaves per plant line, taken from three or more individual plants, with two inoculations per leaf. Error bars represent SEM; letters denote significant difference ($p<0.001$, one-way ANOVA with Holm-Sidak). (b) Representative leaf images showing increased lesion size of transgenic tdTomato expressing *P. infestans* isolate 88069 (McLellan *et al*. 2013) on 35S:AVR2 potato compared to untransformed WT potato. Images are taken under UV light.

As expression of AVR2 in potato[1] increased susceptibility, we predicted that transient expression in *N. benthamiana* would also enhance *P. infestans* colonisation. *N. benthamiana* is a model solanaceous host for *P. infestans* that has been extensively used in RXLR

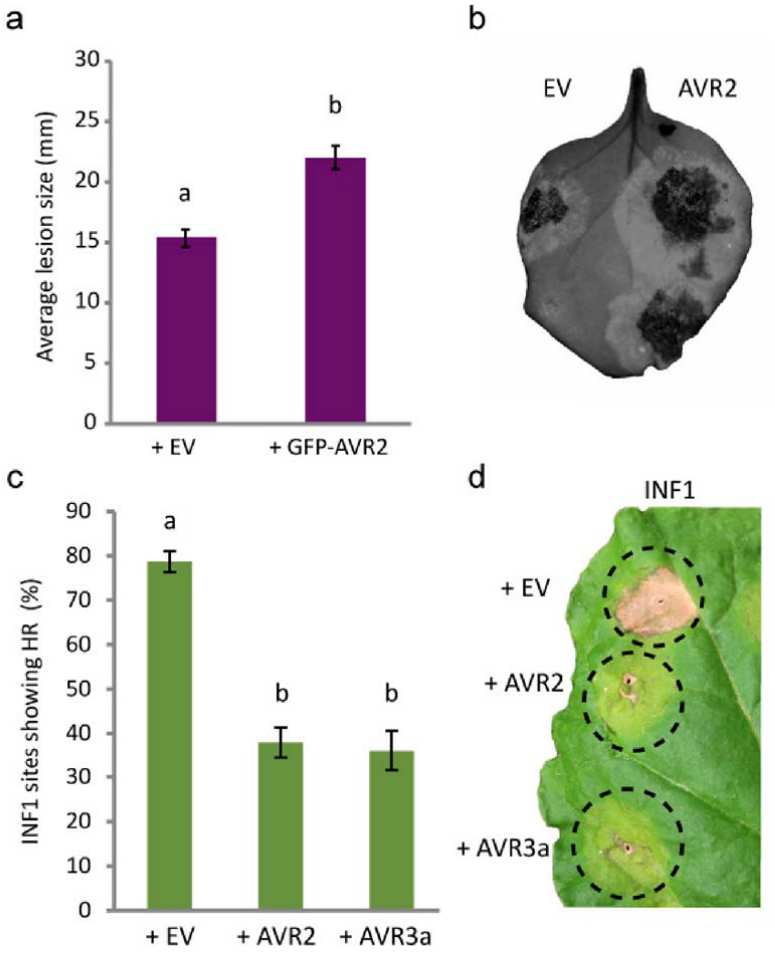


Figure 5 AVR2 negatively regulates immunity to *P. infestans,* and suppresses INF1 cell death. (a) Average lesion size (diameter) of *P. infestans* 88069 colonisation on *N. benthamiana*, inoculated 24 hours after *Agrobacterium*-mediated transient expression of GFP-PiAVR2 or empty vector (EV) control. Results combine 3 biological reps, consisting of at least 6 plants each with 6 infiltrations per construct. Error bars show SEM; letters denote significant difference ($p \leq 0.001$) using one-way ANOVA (Holm-Sidak). (b) Representative leaf images showing increased *P. infestans* 88069 lesion size with GFP-AVR2 expression. Images were taken under UV light to show full extent of infection. (c) Percentage of inoculation sites leading to cell death following co-expression of INF1 with AVR2, AVR3a (positive control), or an empty vector (negative control) in *N. benthamiana*. Error bars show SEM; letters denote significant difference ($p \leq 0.001$) using one-way ANOVA (Holm-Sidak). Results combine at least 3 experimental replicates, consisting of 4 or more plants, with 3 or more infiltrations per plant, per combination. (d) Representative leaf image showing suppression of INF1-triggered cell death when AVR2, or AVR3a are co-expressed

effector research as it facilitates transient expression, cell biology and silencing studies (Whisson et al., 2016). AVR2 was transiently expressed using Agrobacterium, infiltration sites were subsequently inoculated with *P. infestans* sporangia, and disease progression

281 monitored. Transient AVR2 expression resulted in significantly increased lesion sizes (Figure
282 5a, b). This observation is consistent with the stable expression of AVR2 in *S. tuberosum*
283 which also enhanced *P. infestans* leaf colonisation (Figure 4a, b) confirming that AVR2
284 enhances late blight susceptibility in both plant systems.

285 A major virulence strategy of pathogens is to suppress aspects of plant immunity, impairing
286 host defences to increase the chance of a successful infection. INFESTIN1 (INF1) is an
287 oomycete elicitin that is recognised as a PAMP by PRRs in *N. benthamiana* and a number of
288 *Solanum* species, triggering an immune response that results in localised cell death in a
289 BAK1-dependent manner (Du et al., 2015; Chaparro-Garcia et al., 2011). This can be used
290 as a phenotypic read-out for effective PTI in *N. benthamiana* and is already known to be
291 suppressed by *P. infestans* effector AVR3a (Bos et al., 2010). Agrobacterium expressing
292 INF1 was co-infiltrated with Agrobacterium expressing AVR2 and PiAVR3a as a positive
293 control. Expression of AVR2 suppressed INF1-mediated cell death to a similar extent as the
294 PiAVR3a control (Figure 5c, d). These results demonstrate that AVR2 plays a role in
295 attenuating plant immunity. This is a key role of pathogen effectors, and a crucial part of the
296 'zig-zag' model of molecular plant-pathogen interactions (Jones and Dangl, 2006).

297

298 **Transient overexpression of StCHL1 suppresses INF1-triggered cell death and**
299 **enhances *P. infestans* colonisation in *N. benthamiana***

300 Of the BR-induced genes that are constitutively up-regulated in 35S:AVR2 transgenic potato
301 lines, StCHL1 (bHLH7) was of particular interest as a homologue of bHLH domain containing
302 proteins shown to regulate crosstalk between growth and immunity in Arabidopsis (Fan et
303 al., 2014; Malinovsky et al., 2014) (Supplementary Figure S3 and S4) . StCHL1 was one of
304 the most strongly up-regulated genes following BR treatment of potato, with expression 13-
305 fold higher than untreated plants (Figure 2c). StCHL1 was thus taken forward for functional
306 analysis to determine any impact on immunity in solanaceous plants. Transient co-

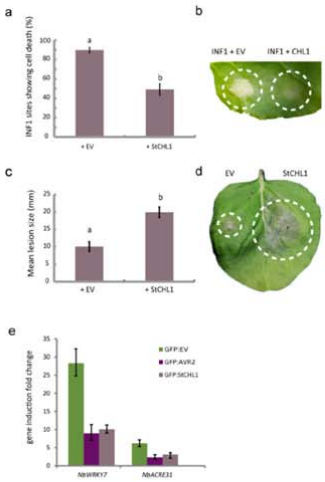


Figure 6 StCHL1 suppresses immunity and increases susceptibility to *P. infestans* in *N. benthamiana*. (a) Graph shows percentage of leaf infiltration sites at 5 dpi resulting in cell death following *Agrobacterium*-mediated co-expression of INF1 with either StCHL1 or an empty vector (EV) control. Error bars show SEM, a≠b ($p \leq 0.001$) in one way ANOVA (Holm-Sidak). Results are combined from 4 experimental replicates consisting of at least 4 plants, each, with at least 6 infiltrations per plant per expression combination. (b) Representative leaf image showing suppression of INF1 cell death when CHL1 is co-expressed. (c) StCHL1 or an empty vector control were transiently expressed in *N. benthamiana*. Sites were inoculated with *P. infestans* 88069 sporangia suspension 24 hours later, with lesions measured (diameter in mm) at 7dpi. Error bars show SEM; letters denote significant difference ($p \leq 0.001$ in one way ANOVA, Holm-Sidak). Results are combined from 4 experimental replicates. (d) Representative leaf image showing increased *P. infestans* colonisation following StCHL1 expression in *N. benthamiana*. (e) PTI marker gene (*NbWRKY7, NbACRE31*) expression in *N. benthamiana* 1 h after *P. infestans* culture filtrate treatment, relative to untreated plants (which were given a value of 1) by qRT-PCR. Treatment occurred 2 days after *Agrobacterium*-mediated transient expression of PiAVR2, StCHL1, or an empty vector control. Expression levels were normalised to *NbEF1a*. Data represents the average of three technical replicates, each from 2 experimental replicates combined, +/- SD.

expression of Agrobacterium delivered StCHL1 (Supplementary Figure S5) with the PAMP INF1 resulted in suppression of INF1-triggered cell death, indicating compromised PTI (Figure 6a,b). In addition, transient expression of StCHL1 attenuated the induction of the PTI

marker genes *NbWRKY7* and *NbACRE31* upon treatment with *P. infestans* CF, further demonstrating that StCHL1 antagonises immunity. AVR2 expression also negatively affected PTI marker gene induction in a similar manner (Figure 6c). Finally, when StCHL1 was transiently over-expressed *P. infestans* leaf colonisation of *N. benthamiana* was significantly enhanced, consistent with previous reports that its homologues HBI1 and CIB1 act as negative regulators of immunity (Malinovsky et al., 2014, Fan et al., 2014) (Figure 6d,e). These results suggest that StCHL1, like Arabidopsis CIB1 and HBI1 TFs, represents an important node of crosstalk between BR signalling and PTI in solanaceous plants, acting to suppress the latter.

VIGS of *NbCHL1* in *N. benthamiana* reduces *P. infestans* colonisation and attenuates AVR2 suppression of INF1-triggered cell death

We identified the *N. benthamiana* orthologue of StCHL1 (Supplementary Figure S3 and S4) and designed two Virus-Induced Gene Silencing (VIGS) constructs (5' and 3') to silence it (Supplementary Figure S6a). Expression of *NbCHL1* VIGS constructs in *N. benthamiana* resulted in dwarfed plants with some mildly curled leaves reminiscent of plants with perturbed BR signalling (Supplementary Figure S6b). *NbCHL1* transcript was reduced by 40-60 % in 3 biological replicates of plants expressing either VIGS construct (Supplementary Figure S6c). We inoculated detached leaves from *NbCHL1* (5' and 3' constructs) silenced plants with *P. infestans* sporangia, and disease progression was monitored for 6-7 days in four biological replicates. Silencing of *NbCHL1* caused a significant decrease of *P. infestans* colonisation measured by both sporangia counting and lesion diameter (Figure 7a, b). Critically, we observed a significant reduction in the ability of AVR2 to suppress INF1-triggered cell death in the *NbCHL1* VIGS plants, whereas suppression by the control effector AVR3a was unaltered. The failure to completely attenuate cell death suppression by AVR2 could be due to the low silencing efficiency of the VIGS constructs.

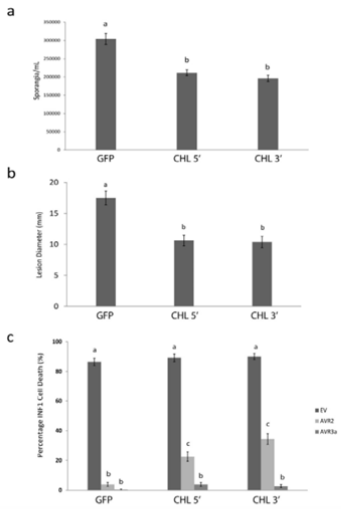


Figure 7 Silencing of NbCHL1 in *N. benthamiana* compromises *P. infestans* infection and perturbs the ability of PiAVR2 to suppress INF1 cell death. a) Silencing of NbCHL1 using two independent VIGS constructs (TRV:NbCHL1 5' and TRV:NbCHL1 3') significantly reduced (one-way ANOVA, P<0.001, n= 136) sporulation of pathogen on NbCHL1 silenced *N. benthamiana* compared to TRV:GFP control; b) *P. infestans* 88069 lesion diameter also showed a significant reduction (one-way ANOVA, p≤0.05, n=223) compared to TRV:GFP control. c) The graph shows the percentage of inoculation sites leading to cell death following co-expression of INF1 with AVR2, AVR3a (positive control), or an empty vector (negative control) in CHL1 silenced *N. benthamiana* and GFP control. Knockdown of *NbCHL1* transcript compromised the ability of PiAVR2 to attenuate INF1 HR. A significant increase is seen in INF1 HR in TRV:NbCHL1 5' and TRV:NbCHL1 3' plants (one-way ANOVA, p≤0.001, using 41 biological replicates and at least 6 inoculations per replicate), compared with the TRV:GFP control at 5 dpi. No significant change is seen in AVR3a suppression of INF1 cell death between the NbCHL1 silenced and GFP control plants. The significance is denoted by lowercase letters and Error bars shown represent SEM.

A common mode-of-action for phytopathogen effectors is to reduce or inhibit activity of their host targets (Rovenich et al., 2014). The enhanced *P. infestans* pathogenicity facilitated by AVR2 expression, in combination with its suppressive effect on PTI, does not support a

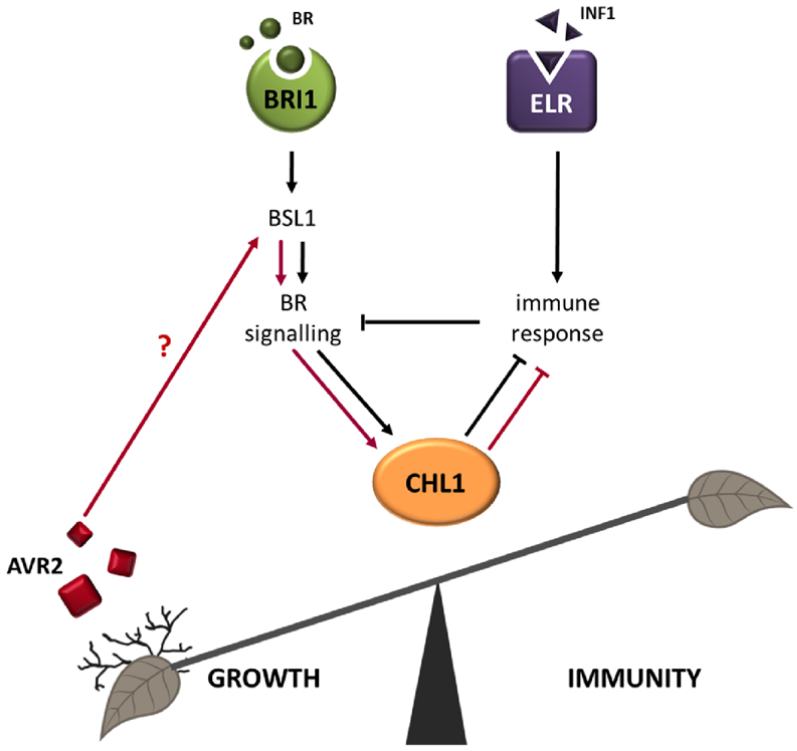


Figure 8: Proposed model indicating how PiAVR2 tips the balance between growth and immunity to promote potato late blight disease. Perception of brassinosteroid (BR) by the receptor BRI1 activates the BR signalling pathway, inducing CHL1 (black arrows) which is proposed to stimulate growth and development. Conversely, we show that CHL1 suppresses immunity triggered by perception of the oomycete PAMP INF1 by receptor ELR. Transgenic potato plants expressing AVR2 lead to activation of the BR signalling pathway and up-regulation of CHL1 to suppress immunity (red arrows). We propose that AVR2 activates BR signalling by stimulating BSL1 activity (red question mark).

model in which AVR2 inhibits the BR[1] pathway. Indeed, it demonstrates the opposite to be true; the pathogen benefits from the role of effector AVR2 in activating the BR pathway, resulting in the upregulation of CHL1 to act as a negative regulator of immunity (Figure 8).

We predict that if assays in this paper were repeated with known activators of the BR pathway e.g. the GSK3 inhibitor, Bikinin (De Rybel et al., 2009) the effect on INF1 cell death, *P. infestans* growth and BR marker gene expression would be similar to expression of AVR2.

A number of examples highlight that the presence and activity of host effector targets can be required for host susceptibility (Yang et al., 2016; Boevink et al., 2016a; Wang et al., 2015; Cui et al., 2010; Chen et al., 2010). Plant genes that are required to support infection, often acting as negative regulators of immunity, are known as susceptibility (S) factors (van Schie and Takken, 2014; Boevink et al., 2016b). The mutation or knockdown of S factors limits the ability of the pathogen to cause disease. When S factors are direct effector targets, their presence is required for manipulation by effector activity to promote disease. Here, silencing of *NbCHL1* compromised susceptibility of *N. benthamiana* to *P. infestans* and the ability of AVR2 to suppress INF1-triggered cell death, implying it should be considered an S factor and could be an indirect target of AVR2 activity to increase host susceptibility (van Schie and Takken, 2014).

In conclusion, this work identifies AVR2 as the first effector from a filamentous plant pathogen to exploit the antagonistic crosstalk between brassinosteroid signalling and innate immunity. This represents a novel, indirect mode of innate immune suppression by a pathogen effector. Future work will focus on the mechanism by which AVR2 promotes BR pathway activity to the benefit of the pathogen and to examine the how this activity is perceived by R2-like NB-LRRs. In particular, given that AVR2 promotes the BR pathway, future work will determine the precise relationship between this effector and its target, the candidate phosphatase BSL1, which can be regarded as a likely positive regulator of BR signalling (Figure 8). The intricacies of cross-talk between growth and innate immunity in plants raise a crucial point for breeding efforts: a push towards one may be at the expense of the other. This highlights a need for balance, and to maintain a whole-plant view towards optimising both yield and disease resistance in our agricultural systems.

Materials and Methods

Agrobacterium-mediated transient expression Constructs used in this work were transformed by electroporation into the Agrobacterium strain Agl1 VirG pSOUP. Liquid YEB were inoculated using bacteria from fresh plates and incubated overnight at 28°C with shaking. Cultures were spun down at 3000 rpm for 10 minutes, and the bacterial pellet resuspended in 10 mM MES 10 mM $MgCl_2$ buffer. OD_{600} was adjusted to 0.5 for cell death assays, or to 0.1 for *P. infestans* colonisation assays, with acetosyringone added at 200 µM. Leaves of *N. benthamiana* or *S. tuberosum* cv. Desiree were infiltrated on the abaxial surface, using a 1 ml syringe after wounding with a needle. INF1 cell death suppression assays were performed as previously described (Gilroy et al., 2011b).

***P. infestans* colonisation** *P. infestans* strain 88069 expressing tdTomato fluorescent protein (McLellan et al., 2013, Saunders et al., 2012) was grown on rye agar supplemented with 20 µg/ml geneticin (Thermo Fisher Scientific Ltd.) as a selective antibiotic. To harvest sporangia, 10 day old plates were flooded with 5 ml sterile distilled water before scraping with a spreader onto a 70 µM cell strainer placed on a 50 ml falcon tube. The suspension containing sporangia was spun down at 3000 rpm for 10 minutes, after discarding supernatant, the pellet was resuspended in sterile distilled water. Sporangia were quantified using a haemocytometer, and adjusted to a concentration of 50,000 ml^{-1}. 10 µl droplets were pipetted onto the abaxial surface of detached leaves, maintained in sealed boxed with moist tissue. Boxes were kept in darkness for the first 24 hours to reduce UV degradation of sporangia. Lesions were measured at the widest point 7 days post infiltration. When used in combination, *P. infestans* was inoculated 24 hours after infiltration with Agrobacterium suspension. Lesions were measured at the widest point 7 days post inoculation. Disease scoring data and INF-mediated cell death suppression assay data (see above) were subjected to statistical analysis using one-way ANOVA (Holm-Sidak) in Sigmaplot (Systat Software Inc.)

396 **Western Blot** Leaf tissue samples were taken 48 hours post-infiltration with Agrobacterium
397 suspensions, and immediately frozen in liquid nitrogen. Protein extraction was carried out by
398 boiling ground leaf tissue samples in 2x SDS sample buffer with 20mM DTT (Sigma-Aldrich
399 Co.) at 95 °C for 10 minutes, followed by centrifugation at 13,000 rpm for 5 minutes.
400 Samples were separated on 4-12 % Bis-Tris PAGE gels with MES buffer using an X-Blot
401 Mini Cell (all Thermo Fisher Scientific Inc.), followed by transfer to nitrocellulose membrane
402 (Amersham Protran premium 0.45 µm NC, GE Healthcare Life Sciences) using an X10 Blot
403 Module (Thermo Fisher Scientific inc.) following manufacturers' instructions. Membranes
404 were then stained using Ponceau solution to visualise relative protein loading. Membranes
405 were blocked in 4 % milk in 1 x phosphate-buffered saline with 0.1% Tween (1xPBS-T) by
406 shaking overnight at 4 °C. Membranes were then incubated for 2 hours with a polyclonal
407 GFP antibody raised in rabbit (Insight Biotechnology) at 1:1000 in 4% milk 1xPBST, before
408 washing 3 x 5 (what I did was 3 x 10) minutes in 1x PBST. A secondary incubation with anti-
409 rabbit IgG HRP (Sigma-Aldrich Co.) at 1:5000 was carried out for 50 minutes, before a
410 further 6 x 5 minute washes. Signal was detected using Amersham ECL Prime as described
411 in the manufacturers' instructions, on Amersham Hyperfilm ECL film (both GE Healthcare
412 Life Sciences). Films were developed with a Compact X4 Automatic Processor (Xograph
413 Healthcare Ltd.)

414 **Virus-induced gene silencing (VIGS)** Virus-induced gene silencing (VIGS) constructs
415 consisted of approximately 250 bp PCR fragments of the gene targeted for silencing, cloned
416 into pBinary Tobacco Rattle Virus (TRV) vectors (Liu et al., 2002) (Supplementary Figure 6).
417 A TRV construct expressing a fragment of GFP was used as a control (Gilroy et al., 2007)
418 and two constructs for silencing *NbCHL1* are described (Supplementary Figure 6). To
419 achieve transient silencing, *N. benthamiana* plants at the four-leaf stage were pressure
420 infiltrated with a mixture of Agrobacterium strain AGL1 containing TRV RNA1 at a final OD_{600}
421 of 0.25, and the fragment corresponding to the gene of interest, at a final OD_{600} of 0.5. The

two largest leaves were infiltrated fully, and viral infection allowed to develop for 2-3 weeks before the plants were used in experiments.

Plant Material *N. benthamiana* and *S. tuberosum* cv Desiree plants were grown in general purpose compost, under long-day glasshouse conditions of 16 hours light at 22 °C, light intensity of 130–150 μE m^{-2} s^{-1} and 40 % humidity unless otherwise stated. *N. benthamiana* was used for *Agrobacterium* infiltrations/*P. infestans* colonisation at 4-5 weeks old, with *S. tuberosum* at 6-8 weeks old.

Generation of 35S:AVR2 transgenic potato cv. Desiree AGL1 *Agrobacterium* culture (OD_{600} 0.8) containing 35S:AVR2 in pGRAB vector (Gilroy et al., 2011a) and 100 mM acetosyringone was added to 25 ml MS30 liquid medium and co-cultivated with internodes from 4-week old potato cv. Desiree tissue culture plants for 20 min at 24 °C/50 rpm in the dark. Explants were blot dried and transferred to solid LSR1 medium (Kumar, 1995) for 3 days, then subsequently transferred weekly on fresh LSR1 medium containing 320 mg/L timentin and 2.5 mg/L phosphinothricin for a further 4-6 weeks to develop callus at 18 °C +/- 2 °C and 16 hr light. Explants with well developed callus were transferred to LSR2 medium (Kumar, 1995) until shoots developed. Independent shoots were screened by PCR using BAR primers to confirm the presence of the transgene. Transgenic shoots were micropropagated on MS30 medium before being transferred to the glasshouse.

Quantification of stomata Epidermal leaf prints were obtained by pressing leaf sections onto microscope slides covered with transparent adhesive tape treated with acetone (Nagel et al., 1994). A compound microscope was used to view the epidermal leaf prints, with number of stomata and number of epidermal cells counted per 0.5 mm^2 area. Multiple prints were scored, representing at least 3 leaves per plant across 3 plants or more. Stomata percentage was calculated as [no. of stomata/ (no. of stomata + no. of epidermal cells)]*100 as previously described (Ogaya et al., 2011). For confocal microscopy, leaf tissue was first stained with Calcofluor White (Sigma-Aldrich) for 10 minutes before mounting sections on a

448 microscope slide. Images were acquired on a Zeiss 710 confocal microscope with a Zeiss
449 Epiplan APO X20/0.6 lens using 405 nm excitation and collecting emissions between (417
450 and 480 nm).

451 **Hormone/PTI elicitor treatment** Epibrassinolide (EBL) (Sigma-Aldrich) was first solubilised
452 at 20 mM in ethanol. EBL treatment was carried out by foliar spray at 50 µM in distilled
453 water, with the addition of 0.5% Tween-20 (Sigma-Aldrich Co.). Distilled water with ethanol
454 and Tween-20 was used as a negative control. *P. infestans* culture filtrate (CF) was
455 prepared by inoculation of sterile amended lima bean (ALB) broth with *P. infestans* strain
456 88069, left to incubate in darkness at room temperature for 5 days before filtering the
457 solution through 70 µm nylon mesh (BioDesign CellMicroSieves; Fisher Scientific UK) to
458 remove mycelium. Culture filtrate was filter sterilized through a 0.20 µm syringe filter
459 (Millipore, UK). This was used to pressure infiltrate leaves of *S. tuberosum* cv. Desiree, or *N.*
460 *benthamiana* by wounding lightly with a needle before infiltrating with a 1 ml syringe.
461 Uninoculated ALB broth was used as a control. Flg22 peptide (Peptide Protein Research
462 Ltd.) was dissolved at 40 µM in sterile distilled water before infiltration of leaves in the same
463 manner.

464 **Gene Expression Analysis** RNA was isolated from plant tissue with the RNeasy Plant Mini
465 Kit (Qiagen) according to the manufacturers' instructions, including the on-column DNase
466 treatment. RNA was quantified using a Nanodrop 1000 (Thermo Scientific) and cDNA
467 synthesised using Superscript II (Qiagen) with oligo dT primers (Eurofins MWG Operon).
468 QRT-PCR was performed using Maxima SYBR green qPCR Mastermix (Thermo Scientific).
469 Detection and data acquisition was achieved with a Chromo4^{TM} real-time detector with MJ
470 Research PTC-200 thermal cycler and Opticon Monitor 3.1.32 software (all Bio-Rad
471 Laboratories Inc.). Reactions were incubated at 95 °C for 15 minutes, before 40 cycles of 95
472 °C for 15 seconds, 60 °C for 1 minute, and plate reading. A melting curve was added
473 between 58 °C and 95 °C, with plate read every 1 °C and hold for 5 seconds. Data was
474 analysed using the ΔΔCt method (McLellan et al., 2013) with expression normalised to a

housekeeping gene (Ubiquitin for *S. tuberosum*, or Elongation Factor 1α for *N. benthamiana*). All primers (Eurofins MWG operon) are shown in Supplementary Table 5. Primer design was based on sequence information from Sol Genomics Network (Fernandez-Pozo et al., 2015) at www.solgenomics.net, and facilitated by the use of Primer3 (Untergrasserat et al., 2012, Koressaar and Remm, 2007) http://primer3.ut.ee/ and NetPrimer sotware (PREMIER Biosoft, USA) at http://www.premierbiosoft.com/netprimer/.

Microarray analysis *S. tuberosum* cv. Desiree plants were grown from tubers under controlled conditions with 16 hours of light at 18 °C and 0 % humidity. Epibrassinolide (Sigma) was first solubilised at 20 mM in ethanol before dilution in distilled water. Six-week old plants were sprayed with a fine mist of 50 µM Epibrassinolide or a mock control containing distilled water and ethanol only. Leaf tissue was collected 3 h and 24 h after EBL/mock treatment and immediately frozen in liquid nitrogen, with three compound leaves harvested for each treatment at each timepoint. Material from four biological replicates was taken forward for microarray analysis. RNA extraction was carried out as above, with sample integrity assessed using a Bioanalyzer (Agilent Technologies). RNA was labelled using the Agilent Two Colour Low Input Quick Amp Labelling Kit (v 6.5; as recommended) and, following purification, cRNA hybridised to custom JHI *Solanum tuberosum* 60K microarrays (ArrayExpress accession A-MEXP-2272) overnight. Arrays were washed and scanned using an Agilent G250 5B scanner, prior to data extraction using Agilent FE software and analysis in GeneSpring (v 7.3 Agilent Technologies; ArrayExpress data accession E-MTAB-3854). Data were normalised using default Lowess settings prior to re-importing into Genespring as individual samples. Filtering was performed to remove those probes with no detectable expression and statistically significant gene expression between treatments was identified using volcano filtering (T-test p-value <0.05; fold-change >2x).

Constructs and cloning StCHL1 was synthesised with Gateway sites (Eurofins Scientific) and recombined into the entry vector pDONR201 using BP clonase (Invitrogen), followed by recombination into the GFP-tagged vector pB7WGF2 (Karimi et al., 2002) using LR clonase

(Invitrogen). GFP-AVR2 and pGRAB-AVR2 were cloned from *Phytophthora infestans* as previously described (Gilroy et al., 2011a), as were 35S-INF1 and GFP-AVR3a (Gilroy et al., 2011b).

Sequence analysis and gene ontology Functional categories were assigned to *S. tuberosum* transcripts using Mapman (Thimm et al., 2011). BLAST analysis and sequence acquisition from The Arabidopsis Information Resource (TAIR) (Lamesch et al., 2012) and the *Solanum* Genome Network (Fernandez-Pozo et al., 2015). Protein domain prediction was facilitated by the Pfam protein families database (Finn et al., 2014). Bayesian (MrBayes) tree was generated using Topali v2.5 on full length amino acid sequences (Biomathematics and Statistics Scotland).

FIGURES

Figure 1 Transgenic potato cv. Desiree lines expressing 35S:Avr2 show morphological hallmarks of an overactive BR pathway. (a) Growth morphology of 35S:AVR2 plants (#29 and #39) showing twisted stems and curled leaves, compared to untransformed potato cv. Desiree (WT). (b) Leaf phenotype of 35S:AVR2 plants showing reduced compound formation and loss of symmetry. (c) Reduced percentage of stomata in 35S:AVR2 potato plants. Stomata count was expressed as % of total epidermal cells counted per 500 µm. Results combine three biological replicates, each consisting of epidermal leaf prints from three or more plants. Error bars indicate SEM; letters denote significant difference ($p<0.001$ in one-way ANOVA, Holm-Sidak). (d) Confocal microscopy showing reduced stomatal frequency in 35S:AVR2 potato, and enlargement of stomata relative to WT plants. Images are of representative leaves stained with calcofluor white. Scale bar = 100um

Figure 2 Microarray analysis of brassinosteroid (EBL treatment) response in potato (*Solanum tuberosum*) cv. Desiree. (a) Microarray validation by qRT-PCR of two independent biological replicates plotted in one graph. Fold-change from microarray data plotted against fold-change from qRT-PCR for 5 selected BR marker genes examined at 24 hpt. Fold-change log2 transformed to allow symmetry of up and down-regulation. Linear regression was used to determine a co-efficient of determination (R^2). (b) Table of selected marker genes showing significant differential expression with BR treatment. Fold-change values are shown from the microarray data, qRT-PCR validation, and an independent

biological replicate (c) Relative expression of brassinosteroid-regulated genes in untreated potato cv. Desiree (WT; given a value of 1), WT at 24 h after treatment with EBL; and constitutive levels of expression in 35S:AVR2 potato plants, assessed by qRT-PCR. Expression was normalised to *StUbi* and shown relative to WT untreated plants. Graph shows the average of three technical replicates +/- standard deviation, with similar trend observed in two independent biological replicates.

Figure 3 Brassinosteroid-responsive genes are suppressed by PTI. (a) Treatment of potato cv. Desiree with *P. infestans* culture filtrate (CF) results in transcript accumulation of PTI marker genes *StWRKY7* and *StACRE31* by 1 hour after treatment (CF1h), but reduced transcript abundance of BR (EBL)-induced genes *StCHL1, StEXP8, StSAUR67, StCAB50* and *StP69F,* and of brassinosteroid biosynthesis-associated genes *StDWF4* and *StSTDH*. (b) Treatment of potato cv. Desiree with the bacterial PAMP flg22 results in the same opposing patterns of transcript abundance by 1 hour after treatment (F1h) for PTI, BR and brassinosteroid biosynthesis markers as observed in (a). Error bars represent SD across 3 technical replicates, with similar trend observed in two independent biological replicates.

Figure 4 AVR2 expression in potato results in increased susceptibility to *P. infestans*. (a) Lesion size of transgenic *P. infestans* isolate 88069 expressing tdTomato (McLellan *et al*. 2013) (diameter in mm) on 35S:AVR2 potato at 7 days post inoculation of sporangia suspension. Data shown combines 2 independent replicates, each comprising 10 or more leaves per plant line, taken from three or more individual plants, with two inoculations per leaf. Error bars represent SEM; letters denote significant difference ($p<0.001$, one-way ANOVA with Holm-Sidak). (b) Representative leaf images showing increased lesion size of transgenic tdTomato expressing *P. infestans* isolate 88069 (McLellan *et al*. 2013) on 35S:AVR2 potato compared to untransformed WT potato. Images are taken under UV light.

Figure 5 AVR2 negatively regulates immunity to *P. infestans,* and suppresses INF1 cell death. (a) Average lesion size (diameter) of *P. infestans* 88069 colonisation on *N. benthamiana*, inoculated 24 hours after *Agrobacterium*-mediated transient expression of GFP-PiAVR2 or empty vector (EV) control. Results combine 3 biological reps, consisting of at least 6 plants each with 6 infiltrations per construct. Error bars show SEM; letters denote significant difference ($p\leq0.001$) using one-way ANOVA (Holm-Sidak). (b) Representative leaf images showing increased *P. infestans* 88069 lesion size with GFP-AVR2 expression. Images were taken under UV light to show full extent of infection. (c) Percentage of inoculation sites leading to cell death following co-expression of INF1 with AVR2, AVR3a (positive control), or an empty vector (negative control) in *N. benthamiana*. Error bars show SEM; letters denote significant difference ($p\leq0.001$) using one-way ANOVA (Holm-Sidak). Results combine at least 3 experimental replicates, consisting of 4 or more plants, with 3 or more infiltrations per plant, per combination. (d) Representative leaf image showing suppression of INF1-triggered cell death when AVR2, or AVR3a are co-expressed

Figure 6 StCHL1 suppresses immunity and increases susceptibility to *P. infestans* in *N. benthamiana*. (a) Graph shows percentage of leaf infiltration sites at 5 dpi resulting in cell death following *Agrobacterium*-mediated co-expression of INF1 with either StCHL1 or an empty vector (EV) control. Error bars show SEM, $a\neq b$ ($p\leq0.001$) in one way ANOVA (Holm-

Sidak). Results are combined from 4 experimental replicates consisting of at least 4 plants, each, with at least 6 infiltrations per plant per expression combination. (b) Representative leaf image showing suppression of INF1 cell death when CHL1 is co-expressed. (c) StCHL1 or an empty vector control were transiently expressed in *N. benthamiana*. Sites were inoculated with *P. infestans* 88069 sporangia suspension 24 hours later, with lesions measured (diameter in mm) at 7dpi. Error bars show SEM; letters denote significant difference ($p \leq 0.001$ in one way ANOVA, Holm-Sidak). Results are combined from 4 experimental replicates. (d) Representative leaf image showing increased *P. infestans*

colonisation following StCHL1 expression in *N. benthamiana*. (e) PTI marker gene (*NbWRKY7, NbACRE31*) expression in *N. benthamiana* 1 h after *P. infestans* culture filtrate treatment, relative to untreated plants (which were given a value of 1) by qRT-PCR. Treatment occurred 2 days after *Agrobacterium*-mediated transient expression of PiAVR2, StCHL1, or an empty vector control. Expression levels were normalised to *NbEF1a*. Data represents the average of three technical replicates, each from 2 experimental replicates combined, +/- SD.

Figure 7 Silencing of NbCHL1 in *N. benthamiana* compromises *P. infestans* infection and perturbs the ability of PiAVR2 to suppress INF1 cell death. a) Silencing of NbCHL1 using two independent VIGS constructs (TRV:NbCHL1 5' and TRV:NbCHL1 3') significantly reduced (one-way ANOVA, $P<0.001$, n= 136) sporulation of pathogen on NbCHL1 silenced *N. benthamiana* compared to TRV:GFP control; b) *P. infestans* 88069 lesion diameter also showed a significant reduction (one-way ANOVA, $p \leq 0.05$, n=223) compared to TRV:GFP control. c) The graph shows the percentage of inoculation sites leading to cell death following co-expression of INF1 with AVR2, AVR3a (positive control), or an empty vector (negative control) in CHL1 silenced *N. benthamiana* and GFP control. Knockdown of *NbCHL1* transcript compromised the ability of PiAVR2 to attenuate INF1 HR. A significant increase is seen in INF1 HR in TRV:NbCHL1 5' and TRV:NbCHL1 3' plants (one-way ANOVA, $p \leq 0.001$, using 41 biological replicates and at least 6 inoculations per replicate), compared with the TRV:GFP control at 5 dpi. No significant change is seen in AVR3a suppression of INF1 cell death between the NbCHL1 silenced and GFP control plants. The significance is denoted by lowercase letters and Error bars shown represent SEM.

Figure 8: Proposed model indicating how PiAVR2 tips the balance between growth and immunity to promote potato late blight disease. Perception of brassinosteroid (BR) by the receptor BRI1 activates the BR signalling pathway, inducing CHL1 (black arrows) which is proposed to stimulate growth and development. Conversely, we show that CHL1 suppresses immunity triggered by perception of the oomycete PAMP INF1 by receptor ELR. Transgenic potato plants expressing AVR2 lead to activation of the BR signalling pathway and up-regulation of CHL1 to suppress immunity (red arrows). We propose that AVR2 activates BR signalling by stimulating BSL1 activity (red question mark).

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616 **SUPPLEMENTAL DATA**

617

618 Supplementary Figure S1 Transgenic potato lines expressing AVR2.

619 Supplementary Figure S2. Epidermal cell size is increased in 35S:AVR2 expressing
620 transgenic potato lines #29 and #39.

621 Supplementary Figure S3: Protein alignment of CHL1 orthologues.

622 Supplementary Figure S4: StCHL1 is closely related to several bHLH DNA binding proteins
623 from Arabidopsis.

624 Supplementary Figure S5: GFP-StCHL1 is stably expressed in *N. benthamiana*.

625 Supplementary Figure S6 *NbCHL1* silencing vector design, plant phenotypes and silencing
626 levels in *N. benthamiana*.

627 Table S1 List of Top 50 Genes Up-regulated in EBL vs Water Sprayed Potato cv. Desiree at
628 3 h

629 Table S2 List of Top 50 Genes Down-regulated in EBL vs Water Sprayed Potato cv. Desiree
630 at 3 h

631 Table S3 List of Top 50 Genes Up-regulated in EBL vs Water Sprayed Potato cv. Desiree at
632 24 h

633 Table S4 List of Top 50 Genes Down-Regulated in EBL vs Water Sprayed Potato cv.
634 Desiree at 24 H

635 Table S5 Primers used in this Study

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Parsed Citations

Albrecht C, Boutrot F, Segonzac C, Schwessinger B, Gimenez-Ibanez S, Chinchilla D, Rathjen JP, de Vries SC, Zipfel C (2012) Brassinosteroids inhibit pathogen-associated molecular pattern-triggered immune signaling independent of the receptor kinase BAK1. Proc. Natl. Acad. Sci. USA 109, 303-308

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bai MY, Fan M, Oh E, Wang ZY(2012).A Triple Helix-Loop-Helix/Basic Helix-Loop-Helix Cascade Controls Cell Elongation Downstream of Multiple Hormonal and Environmental Signaling Pathways in Arabidopsis. Plant Cell 24, 4917-4929

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Belkhadir Y, Jaillais Y, Epple P, Balsemão-Pires E, Dangl JL, Chory J. (2012). Brassinosteroids modulate the efficiency of plant immune responses to microbe-associated molecular patterns. Proc. Natl. Acad. Sci. USA 109, 297-302

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Block A, Alfano JR (2011) Plant targets for Pseudomonas syringae type III effectors: virulence targets or guarded decoys? Curr Opin Microbiol. 14:39-46

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bos JIB, Armstrong MR, Gilroy EM, Boevink PC, Hein I, Taylor RM, Zhendong T, Engelhardt S, Vetukuri RR, Harrower B, Dixelius C, Bryan G, Sadanandom A, Whisson SC, Kamoun S, Birch PR (2010) Phytophthora infestans effector AVR3a is essential for virulence and manipulates plant immunity by stabilizing host E3 ligase CMPG1. Proc. Natl. Acad. Sci. USA 107, 9909-9914

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Boevink PC, Wang X, McLellan H, He Q, Naqvi S, Armstrong MR, Zhang W, Hein I, Gilroy EM, Tian Z, Birch PRJ. (2016a) A Phytophthora Infestans Rxlr Effector Targets Plant Pp1c Isoforms That Promote Late Blight Disease. Nat Commun, 7:10311.

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Boevink PC, McLellan H, Gilroy EM, Naqvi S, He Q, Yang L, Wang X, Turnbull D, Armstrong MR, Tian Z, Birch PRJ. (2016b) Oomycetes Seek Help From The Plant: Phytophthora Infestans Effectors Target Host Susceptibility Factors. Molecular Plant 9: 636-638

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Campos ML, Yoshida Y, Major IT, de Oliveira Ferreira D, Weraduwage SM, Froehlich JE, Johnson BF, Kramer DM, Jander G, Sharkey TD, Howe GA (2016) Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. Nat Commun. 7:12570

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chandran D, Rickert J, Huang Y, Steinwand MA, Marr SK, Wildermuth MC (2014) Atypical E2F transcriptional repressor DEL1 acts at the intersection of plant growth and immunity by controlling the hormone salicylic acid. Cell Host Microbe. 15:506-13

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chaparro-Garcia A, Wilkinson RC, Gimenez-Ibanez S, Findlay K, Coffey MD, Zipfel C, Rathjen JP, Kamoun S, Schornack S (2011)The receptor-like kinase SERK3/BAK1 is required for basal resistance against the late blight pathogen phytophthora infestans in Nicotiana benthamiana. PLoS One. 6:e16608

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, Qu XQ, Guo WJ, Kim JG, Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC, Mudgett MB, Frommer WB (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468: 527-532

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cui H, Wang Y, Xue L, Chu J, Yan C, Fu J, Chen M, Innes RW, Zhou JM (2010) Pseudomonas syringae effector protein AvrB perturbs Arabidopsis hormone signaling by activating MAP kinase 4. Cell Host Microbe 7: 164-175

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

De Rybel B, Audenaert D, Vert G, Rozhon W, Mayerhofer J, Peelman F, Coutuer S, Denayer T, Jansen L, Nguyen L, Vanhoutte I, Beemster GT, Vleminckx K, Jonak C, Chory J, Inzé D, Russinova E, Beeckman T (2009) Chemical inhibition of a subset of Arabidopsis thaliana GSK3-like kinases activates brassinosteroid signaling. Chem Biol. 16:594-604.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

de Wit M, Spoel SH, Sanchez-Perez GF, Gommers CMM, Pieterse CMJ, Voesenek LACJ, Pierik R (2013) Perception of low red:far-red ratio compromises both salicylic acid- and jasmonic acid-dependent pathogen defences in Arabidopsis. Plant J. 75: 90-103
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Derevnina L, Dagdas YF, De la Concepcion JC, Bialas A, Kellner R, Petre B, Domazakis E, Du J, Wu CH, Lin X, Aguilera-Galvez C, Cruz-Mireles N, Vleeshouwers VG, Kamoun S (2016) Nine things to know about elicitins New Phytol. 212:888-895.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Deslandes L, Rivas S (2012) Catch me if you can: bacterial effectors and plant targets. Trends Plant Sci. 17:644-55
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Dou D, Zhou JM (2012) Phytopathogen effectors subverting host immunity: different foes, similar battleground. Cell Host Microbe 12:484-95.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Du J, Verzaux E, Chaparro-Garcia A, Bijsterbosch G, Keizer PLC, Zhou J, Liebrand TWH, Xie C, Govers F, Robatzek, S, van der Vossen EAG, Jacobsen E, Visser RGF, Kamoun S, Vleeshouwers VGAA (2015) Elicitin recognition confers enhanced resistance to Phytophthora infestans in potato. Nature Plants 1, 15034.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Fan M, Bai MY, Kim JG, Wang T, Oh E, Chen L, Park CH, Son SH, Kim SK, Mudgett MB, Wang ZY (2014) The bHLH transcription factor HBI1 mediates the trade-off between growth and pathogen-associated molecular pattern-triggered immunity in Arabidopsis. Plant Cell 26, 828-841.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Feechan A, Turnbull D, Stevens LJ, Engelhardt S, Birch PRJ, Hein I, Gilroy EM (2015) The Hypersensitive Response in PAMP- and Effector-triggered immune responses. Plant Programmed Cell Death, Springer Publishing. Gunawardena AN, McCabe PF. Chpt10: pp 235-268 ISBN: 978-3-21032-2
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Feng F, Yang F, Rong W, Wu X, Zhang J, Chen S, He C, Zhou JM. (2012) A Xanthomonas uridine 5'-monophosphate transferase inhibits plant immune kinases. Nature 485, 114-118
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Fernandez-Pozo N, Menda N, Edwards JD, Saha S, Tecle IY, Strickler SR, Bombarely A, Fisher-York T, Pujar A, Foerster H, Yan A, Mueller LA (2015) The Sol Genomics Network (SGN)—from genotype to phenotype to breeding. Nucleic Acids Research 43, D1036-D1041
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer EL, Tate J, Punta M (2014) Pfam: the protein families database. Nucleic Acids Research 42, D222-D230
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Francisco M, Joseph B, Caligagan H, Li B, Corwin JA, Lin C, Kerwin RE, Burow M, Kliebenstein DJ (2016) Genome Wide Association Mapping in Arabidopsis thaliana Identifies Novel Genes Involved in Linking Allyl Glucosinolate to Altered Biomass and Defense. Front Plant Sci 7:1010
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gendron JM, Liu JS, Fan M, Bai MY, Wenkel S, Springer PS, Barton MK, Wang ZY (2012) Brassinosteroids regulate organ boundary formation in the shoot apical meristem of Arabidopsis. Proc. Natl. Acad. Sci. USA 109, 21152-21157
Pubmed: Author and Title

CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gilroy EM, Breen S, Whisson SC, Squires J, Hein I, Kaczmarek M, Turnbull D, Boevink PC, Lokossou A, Cano LM, Morales J, Avrova AO, Pritchard L, Randall E, Lees A, Govers F, van West P, Kamoun S, Vleeshouwers VG, Cooke DE, Birch PR (2011a) Presence/absence, differential expression and sequence polymorphisms between PiAVR2 and PiAVR2-like in Phytophthora infestans determine virulence on R2 plants. New Phytologist 191, 763-776.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gilroy EM, Hein I, van der Hoorn R, Boevink PC, Venter E, McLellan H, Kaffarnik F, Hrubikova K, Shaw J, Holeva M, López EC, Borras-Hidalgo O, Pritchard L, Loake GJ, Lacomme C, Birch PR (2007) Involvement of cathepsin B in the plant disease resistance hypersensitive response. The Plant Journal 52, 1-13.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gilroy EM, Taylor RM, Hein I, Boevink P, Sadanandom A, Birch PR (2011b) CMPG1-dependent cell death follows perception of diverse pathogen elicitors at the host plasma membrane and is suppressed by Phytophthora infestans RXLR effector AVR3a. New Phytologist 190, 653-666.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Goda H, Shimada Y, Asami T, Fujioka S, Yoshida S (2002) Microarray analysis of brassinosteroid-regulated genes in Arabidopsis. Plant Physiol. 130:1319-34.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, Schroeder JI, Peck SC, Rathjen JP (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc. Natl. Acad. Sci. USA 104, 12217-12222
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Huot B, Yao J, Montgomery BL, He SY (2014) Growth-Defense Tradeoffs in Plants: A Balancing Act to Optimize Fitness. Mol Plant. 7: 1267-1287
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jiménez-Góngora T, Kim SK, Lozano-Durán R, Zipfel C (2015) Flg22-Triggered Immunity Negatively Regulates Key BR Biosynthetic Genes. Front Plant Sci. 6:981
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323-329.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kang S, Yang F, Li L, Chen H, Chen S, Zhang J (2015) The Arabidopsis transcription factor BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1 is a direct substrate of MITOGEN-ACTIVATED PROTEIN KINASE6 and regulates immunity. Plant Physiol. 167:1076-86.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Karimi M, Inzé D, Depicker A (2002) GATEWAY™ vectors for Agrobacterium-mediated plant transformation. Trends in Plant Science 7:193-195
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim TW, Michniewicz M, Bergmann DC, Wang ZY (2012) Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. Nature 482, 419-422
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim TW, Guan S, Burlingame AL, Wang ZY (2011) The CDG1 kinase mediates brassinosteroid signal transduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2. Mol Cell. 43:561-71
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim TW, Guan S, Sun Y, Deng Z, Tang W, Shang JX, Sun Y, Burlingame AL, Wang ZY (2009) Brassinosteroid signal transduction

from cell-surface receptor kinases to nuclear transcription factors. Nat Cell Biol. 11:1254-60.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim TW, Wang ZY (2010) Brassinosteroid signal transduction from receptor kinases to transcription factors. Annu Rev Plant Biol. 61:681-704.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Koressaar T, Remm M (2007) Enhancements and modifications of primer design program Primer3 Bioinformatics 23:1289-9111
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kumar A. (1995) Agrobacterium-mediated transformation of potato genotypes Agrobacterium Protocols: Methods in Molecular Biology 44:121-128
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lamesch P, Berardini TZ, Li D, Swarbreck D, Wilks C, Sasidharan R, Muller R, Dreher K, Alexander DL, Garcia-Hernandez M, Karthikeyan AS, Lee CH, Nelson WD, Ploetz L, Singh S, Wensel A, Huala E (2012) The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. Nucleic Acids Research 40: D1202-1210
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li J, Chory J. (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction Cell 90: 929-938.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lin W, Lu D, Gao X, Jiang S, Ma X, Wang Z, Mengiste T, He P, Shan L. (2013) Inverse modulation of plant immune and brassinosteroid signaling pathways by the receptor-like cytoplasmic kinase BIK1 Proc. Natl. Acad. Sci. USA 110:12114-12119.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liu Y, Schiff M, Marathe R, Dinesh-Kumar SP (2002) Tobacco Rar1, EDS1 and NPR1/NIM1 like genes are required for N-mediated resistance to tobacco mosaic virus. The Plant Journal 30: 415-429.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lo SF Yang SY Chen KT, Hsing YI, Zeevaart JA, Chen LJ, Yu SM (2008) A novel class of gibberellin2-oxidases control semidwarfism, tillering, and root development in rice. The Plant Cell 20, 2603-2618.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lozano-Durán R, Zipfel C (2015) Trade-off between growth and immunity: role of brassinosteroids.Trends Plant Sci. 20:12-9
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lozano-Durán R, Macho AP, Boutrot F, Segonzac C, Somssich IE, Zipfel C (2013) The transcriptional regulator BZR1 mediates trade-off between plant innate immunity and growth Elife 2:e00983.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Malinovsky FG, Batoux M, Schwessinger B, Youn JH, Stransfeld L, Win J, Kim SK, Zipfel C (2014) Antagonistic regulation of growth and immunity by the Arabidopsis bHLH transcription factor HBI1. Plant Physiology 164:1443-1455.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Maselli GA, Slamovits CH, Bianchi JI, Vilarrasa-Blasi J, Caño-Delgado AI, Mora-García S. (2014) Revisiting the evolutionary history and roles of protein phosphatases with Kelch-like domains in plants. Plant Physiol. 164:1527-41
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

McLellan H, Boevink PC, Armstrong MR, Pritchard L, Gomez S, Morales J, Whisson SC, Beynon JL, Birch PR (2013) An RxLR Effector from Phytophthora infestans Prevents Re-localisation of Two Plant NAC Transcription Factors from the Endoplasmic Reticulum to the Nucleus. PLOS Pathog. 9: e1003670.
Pubmed: Author and Title

CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mora-García S, Vert G, Yin Y, Caño-Delgado A, Cheong H, Chory J (2004).Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. Genes and Development. 18:448-460
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Müssig C, Fischer S, Altmann T (2002) Brassinosteroid-regulated gene expression. Plant Physiol. 129:1241-51
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nam K, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 110:203-212.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nguyen HP, Chakravarthy S, Velásquez AC, McLane HL, Zeng L, Nakayashiki H, Park DH, Collmer A, Martin GB (2010) Methods to study PAMP-triggered immunity using tomato and Nicotiana benthamiana. Mol Plant Microbe Interact 23:991-999.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ogaya R, Llorens L, Peñuelas J (2011) Density and length of stomatal and epidermal cells in "living fossil" trees grown under elevated CO2 and a polar light regime. Acta Oecologica 37:381-385.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rovenich H, Boshoven JC, Thomma BPHJ (2014) Filamentous pathogen effector functions: of pathogens, hosts and microbiomes. Current Opinion in Plant Biology 20:96-103
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Saunders DG, Breen S, Win J, Schornack S, Hein I, Bozkurt TO, Champouret N, Vleeshouwers VG, Birch PR, Gilroy EM, Kamoun S (2012) Host Protein BSL1 Associates with Phytophthora infestans RXLR Effector AVR2 and the Solanum demissum Immune Receptor R2 to Mediate Disease Resistance. The Plant Cell 24: 3420-3434
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sreeramulu S, Mostizky Y, Sunitha S, Shani E, Nahum H, Salomon D, Hayun LB, Gruetter C, Rauh D, Ori N, Sessa G (2013) BSKs are partially redundant positive regulators of brassinosteroid signaling in Arabidopsis. Plant J. 74:905-19
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schwessinger B, Roux M, Kadota Y, Ntoukakis V, Sklenar J, Jones A, Zipfel C (2011) Phosphorylation-dependent differential regulation of plant growth, cell death, and innate immunity by the regulatory receptor-like kinase BAK1. PLoS Genet. 7:e1002046.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Spartz AK, Lee SH, Wenger JP, Gonzalez N, Itoh H, Inze D, Peer WA, Murphy AS, Overvoorde PJ, Gray WM (2012) The SAUR19 subfamily of SMALL AUXIN UP RNA genes promote cell expansion. Plant J 70: 978-990
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tang W, Kim TW, Oses-Prieto JA, Sun Y, Deng Z, Zhu S, Wang R, Burlingame AL, Wang ZY (2008) BSKs mediate signal transduction from the receptor kinase BRI1 in Arabidopsis Science. 321:557-60
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M (2011) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. The Plant Journal 37, 914-939
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3 - new capabilities and interfaces. Nucleic Acids Research 40, e115
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

van Schie CC, Takken FL (2014) Susceptibility genes 101: how to be a good host. Annu. Rev. Phytopathol. 52:551-581

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang H, Mao H (2014) On the origin and evolution of plant brassinosteroid receptor kinases J Mol Evol. 78:118-29.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang X, Boevink P, McLellan H, Armstrong M, Bukharova T, Qin Z, Birch PR (2015) A host KH RNA binding protein is a susceptibility factor targeted by an RXLR effector to promote late blight disease. Molecular Plant 8:1385-95
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang W, Wang ZY (2014) At the intersection of plant growth and immunity. Cell Host Microbe. 15:400-402
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang ZY, Seto H, Fujioka S, Yoshida S, Chory J (2001) BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature 410: 380-383.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Whisson S, Boevink P, Wang S, Birch PRJ (2016)The cell biology of late blight disease. Curr Opin Microbiol 34:127-135
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yang DH, Baldwin IT, Wu J (2013) Silencing brassinosteroid receptor BRI1 impairs herbivory-elicited accumulation of jasmonic acid-isoleucine and diterpene glycosides, but not jasmonic acid and trypsin proteinase inhibitors in Nicotiana attenuata. J Integr Plant Biol, 55: 514-26.
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yang L, McLellan H, Naqvi S, He Q, Boevink PC, Armstrong M, Giuliani LM, Zhang W, Tian Z, Zhan J, Gilroy EM, Birch PRJ (2016) Potato NPH2/RPT2-like protein StNRL1, targeted by a Phytophthora infestans effector, is a susceptibility factor. Plant Physiol 171:645-657
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang D, Ye H, Guo H, Johnson A, Lin H, Yin Y (2014) Transcription factors involved in brassinosteroid repressed gene expression and their regulation by BIN2 kinase. Plant Signal Behav. 9:e27849
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhiponova MK, Vanhoutte I, Boudolf V, Betti C, Dhondt S, Coppens F, Mylle E, Maes S, González-García MP, Caño-Delgado AI, Inzé D, Beemster GT, De Veylder L, Russinova E (2013) Brassinosteroid production and signaling differentially control cell division and expansion in the leaf. New Phytol. 197:490-502
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title